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Shorter sleep duration associated with coronary artery calcification

The design of phase IV clinical trials in nephrology has made a decided move toward the use of intermediate outcomes. While this is an advantage in that generally fewer subjects need to be enrolled and followed for shorter periods of time, the inherent caveat lies in the validation of the intermediate outcome as a true surrogate of the hard clinical outcomes such as mortality or cardiovascular events.[1] Many argue that the validation of coronary artery calcification (CAC) among persons with abnormal kidney function is not complete. A new article by King *et al.* provides insight into the investigation of the potential mechanisms of its genesis.

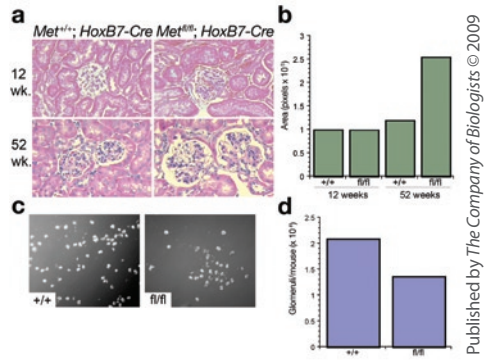
In this observational cohort study, healthy participants from a single site in the Coronary Artery Risk Development in Young Adults (CARDIA) study were included. CAC was measured by computed tomography in 2000–2001 and 2005–2006. Participants had no detectable calcification at baseline, and their incidence of new calcification was the primary outcome. Sleep metrics (wrist actigraphy-measured duration and fragmentation, daytime sleepiness, overall quality, self-reported duration) along with potential confounders (age, sex, race, education, apnea risk, smoking status) and mediators (lipids, blood pressure, body mass index, diabetes, inflammatory markers, alcohol consumption, depression, hostility, self-reported medical conditions) were measured and examined for association with incident calcification. Approximately 12% developed calcification over the 5-year observation period. Longer sleep duration was significantly associated with a decreased risk of new calcification ($P = 0.01$). This association persisted in models adjusted for demographics and for medical history including apnea risk, as well as known mediators of calcification such as lipid and blood pressure parameters. The authors suggest inflammatory and hormonal mediators as causes of both CAC and decreased sleep duration, and decreased sleep duration resulting in transient decreases in glucose tolerance as a cause of CAC. These findings suggest that a renewed interest in sleep may benefit our patients' health in addition to their quality of life. Additionally, we must continue to be objective about our understanding of the pathophysiology of arterial calcification as we scrutinize metrics such as the CAC score as surrogate markers. (*JAMA* 2008; **300**: 2859–2866)

Lynda Szczech

[1] *JAMA* 1999; **282**: 771–778.

Met and the epidermal growth factor receptor cooperatively regulate final nephron number

Kidney development occurs by the interactions between the ureteric bud and the adjacent metanephric mesenchyme. While the ureteric bud branches to form the collecting ducts, the more proximal parts of the nephron develop via mesenchymal–epithelial transformation of the metanephric mesenchyme cells in contact with the tips



Deletion of Met in collecting duct (*Met*$^{fl/fl}$;*HoxB7-Cre*) reduces nephron number. (**a**) Glomeruli at 12 and 52 weeks from *Met*$^{fl/fl}$;*HoxB7-Cre* and *Met*$^{+/+}$;*HoxB7-Cre* (control) mice. (**b**) Quantification of glomerular surface area from kidney sections. (**c**) Representative image of isolated glomeruli from *Met*$^{fl/fl}$;*HoxB7-Cre* and control mice. (**d**) Quantification of total glomeruli per mouse in *Met*$^{fl/fl}$;*HoxB7-Cre* and control.

of ureteric bud branches. The result of this interplay is that the final nephron number is determined by the signals that regulate ureteric bud branching. A number of these signals have been identified, but there is little doubt that more remain to be discovered. During kidney development, Hgf is expressed by the metanephric mesenchyme, whereas its receptor (Met) is present on both ureteric bud and mesenchymal cells. *In vitro* work with neutralizing antibodies against Hgf showed inhibition of ureteric bud branching, suggesting an important role for Hgf. However, loss of either Hgf or Met expression in the mouse led to death by approximately embryonic day 13, and early kidney development appeared normal. Because ureteric bud branching continues through embryonic day 18, the exact role of Hgf–Met signaling in the development of the collecting system and nephron number remained unclear. Ishibe *et al.* now use Cre-loxP technology to address this question. They found that deletion of the Met receptor selectively in the collecting system (*Met*$^{fl/fl}$;*HoxB7-Cre*) did not result in abnormal morphology, but the kidneys of these mice had a 35% reduction in nephron number at 12 weeks of age and glomerular hypertrophy by 1 year of age (Figure). Examination of these collecting ducts revealed sustained upregulation and activation of the Egf receptor. Moreover, addition of Egf to embryonic kidneys (with deleted Met in the ureteric bud) grown *in vitro* rescued the decrease in ureteric bud branching observed *in vivo*. Finally, mice lacking both Met and Egfr signaling in the collecting duct had a marked decrease in ureteric bud branching, small kidneys, renal failure, and early death. Thus, Met and the Egf receptor act cooperatively to regulate branching of the ureteric bud and nephron number. (*Development* 2009; **136**: 337–345; doi:10.1242/10.1242/dev.024463)

Juan Oliver

Vasopressin signaling pathway in primary cilia of renal epithelia

The primary cilium is a solitary, sensory organelle projecting from the apical surface of many cells. Most renal tubular epithelial cells of

the mammalian nephron, with the exception of intercalated cells in the collecting duct, contain a single primary cilium, which responds to fluid flow or mechanical bending by eliciting cytosolic calcium^{2+} signals. The signaling pathways for the regulation of ciliary-located channel function are unknown. In a recent publication, Raychowdhury *et al.* report the presence of type 2 vasopressin receptor (V2R) in primary cilia of LLC-PK$_1$ cells (renal epithelial cells derived from pig kidneys). While hormone receptors in renal tubular epithelial cells have been mostly located to the basolateral membrane of the cells, several receptors, such as V2R and parathyroid hormone receptor, as well as the vasopressin type 1 receptor, have been found in the luminal aspect of tubular epithelia. The function of these luminal receptors remains unclear. The authors demonstrated the presence of V2R in the cilia by the spontaneous fluorescence of a V2R-*gfp* chimera (Figure) and confirmed it by immunocytochemical analysis in LLC-PK$_1$ cells stained with anti-V2R antibodies and in LLC-PK$_1$ cells overexpressing a V2R-Flag chimera, with an anti-Flag antibody. They also demonstrated that ciliary V2R colocalized with adenylyl cyclase in all cell types tested. Finally, they demonstrated functional coupling of the ciliary receptors with adenylyl cyclase by measuring cyclic adenosine monophosphate (cAMP) production in isolated cilia and by testing vasopressin-induced cation-selective channel activity either in reconstituted lipid bilayers or by membrane-attached patch clamping: the addition of either vasopressin (*trans*) or forskolin (*cis*) stimulated cation-selective channel activity in ciliary membranes. These results provide the first demonstration of V2R in primary cilia of renal epithelial cells, and a functional cAMP-signaling pathway, which targets ciliary channel function and may help control the sensory function of the primary cilium. (*Am J Physiol Renal Physiol* 2009; **296**: F87–F97; doi:10.1152/ajprenal.90509.2008)

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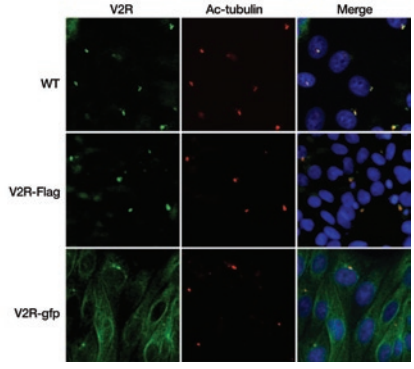

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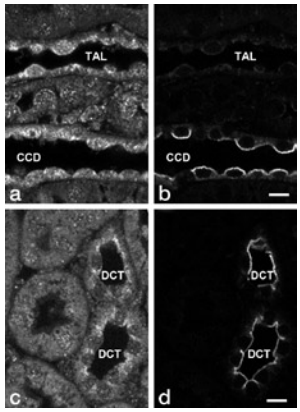
Localization of V2R in primary cilia of renal epithelial cells. V2R was localized to the primary cilia of confluent wild-type LLC-PK$_1$ renal epithelial cells (WT) and further confirmed in cells expressing either the V2R-Flag or the V2R-*gfp* chimera. Cells were also stained with mouse anti-acetylated tubulin antibody (Ac-tubulin; red). DAPI fluorescence (blue) is shown for contrast.

STK39 is a hypertension susceptibility gene

Essential hypertension (EH) is widely believed to involve multiple genes with variant alleles. As with other complex disorders,



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Immunolocalization of SPAK in kidney. (**a**) SPAK strongly localizes to the thick ascending limb (TAL) of the loop of Henle and the cortical collecting duct (CCD). (**b**) Immunolocalization of aquaporin 2 in the same section as in **a** as a segment-specific marker for CCDs. (**c**) Immunolocalization of SPAK in the distal convoluted tubules (DCT). (**d**) Immunolocalization of sodium chloride cotransporter as a segment-specific marker for DCTs.

manifestation of EH in any single individual is likely dependent on a variety of genetic and environment factors, making identification of EH susceptibility genes in the general population a major challenge. In contrast, studies on the genetic basis of rare blood pressure monogenetic disorders have identified mutations in genes affecting a single physiological pathway: renal salt transport. Although such studies highlight the importance of extracellular fluid volume to blood pressure regulation, the underlying genetic basis for EH remains poorly understood. To identify common EH susceptibility genes, Wang *et al.* conducted a genome-wide association analysis in the Old Order Amish, a closed population with descendents from a small number of founders that has a relatively homogeneous lifestyle, making it ideal for identifying genes that underlie complex diseases. They analyzed genotype data for systolic blood pressure and diastolic blood pressure in 542 subjects. This approach identified a novel hypertension susceptibility gene, STK39 (a serine/threonine kinase gene), in which common variants were associated with blood pressure levels. They confirmed this association in an independent Amish and four non-Amish Caucasian samples, including the Diabetes Genetics Initiative, Framingham Heart Study, GenNet, and Hutterites. Functional studies in cells showed that *STK39* interacted with WNK kinases and cation-chloride cotransporters, mutations that are known to cause monogenic forms of blood pressure disorders. The authors also found that *STK39* was expressed in the distal nephron (Figure), where it may interact with these proteins. Although none of the associated single-nucleotide polymorphisms altered protein structure, they identified and experimentally confirmed a highly conserved element with *in vitro* transcription activity as a functional candidate for the association. Thus, variants in *STK39* may influence blood pressure by increasing STK39 expression and consequently altering renal Na^+ excretion, thus unifying rare and common blood pressure-regulating alleles in the same physiological pathway. (*Proc Natl Acad Sci USA* 2009; **106**: 226–231; doi:10.1073/pnas.0808358106)

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